Antibacterial Efficacy of a New Sonic Irrigation Device for Root Canal Disinfection

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Abstract

Introduction: Passive ultrasonic irrigation (PUI) is the most widespread method used to activate irrigation solutions. Concerns have been raised that PUI is less effective in curved root canals and is not passive at all. Our aim was to compare a novel passive sonic irrigation (PSI) device (6000 Hz) with PUI and manual irrigation (MI) with respect to their efficiency in removing different endodontic microorganisms from curved and straight root canals. **Methods:** We performed 2 experiments as follows. In a 3-day infection model, we included 8 groups of single or dual microbial species that were rinsed with 0.9% sodium chloride using PSI, PUI, or MI. Colony-forming units (CFUs) were counted after incubation, and $\log_{10}$ transformations were performed for statistical comparisons. In a 21-d infection model, we tested the same irrigation protocols on 4 groups of microorganisms and used 1.5% sodium hypochlorite as an irrigant. Infection control samples were taken at day 0, 3, 5, and 7 after treatment and were subsequently reincubated. **Results:** Using sodium chloride as an irrigant, the amount of reduction in CFUs compared with the negative control was approximately 3 $\log_{10}$ units for PSI at 6000 Hz, 2 $\log_{10}$ units for PUI, and 1 $\log_{10}$ unit for MI. PSI reduced the microorganism CFUs significantly better than PUI. Using sodium hypochlorite led to a significant reduction in microorganism CFUs even with MI. After 3 days, compared with MI, microorganism regrowth significantly reduced after PSI and PUI treatment, but in these groups, in at least half of the samples, microorganisms were detectable after 7 days. **Conclusions:** PSI at 6000 Hz might be at least equal to PUI with respect to reduction of the microbial load in curved and straight root canals. *(J Endod 2016;■ :1–5)*

Key Words

Disinfection, oral bacteria, root canal, sonic irrigation, ultrasonic irrigation

Significance

Passive sonic irrigation at 6000 Hz seems to perform equal to or better than passive ultrasonic irrigation.

Root canal infections are caused by a variety of mainly anaerobic gram-positive bacteria (1). A persistent intraradicular presence of bacteria after chemomechanical treatment is considered to be a possible cause of endodontic failure (2). Although facultative anaerobes such as *Streptococcus gordonii*, *Fusobacterium nucleatum*, and *Actinomyces oris* have been isolated in primary endodontic infections (3), the persistence of *Enterococcus faecalis* (4) or *Candida albicans* (5) has been associated with persistent periapical lesions and the need for endodontic retreatment. Sodium hypochlorite is considered a suitable disinfecting irrigation solution, but it has been shown that ultrasonic activation of sodium hypochlorite (NaCl) enhances its effectiveness (6). Nowadays, passive ultrasonic irrigation (PUI) seems to be the predominant activation method for endodontic irrigation solutions (7). The main reason for the additional effectiveness via ultrasound has been shown to be caused by acoustic streaming effects that increase wall shear stress and enhance rupturing of intraradicular biofilm (8). However, several limitations have been identified that impose procedural problems when using ultrasonic activation. Wall contact with the oscillating instrument dampens the energy and constrains the file movement (9). Therefore, in curved root canals, ultrasonic instruments are less likely to oscillate freely. It has been shown that even in straight root canals an ultrasonic instrument comes into contact with the wall during at least 20% of the working time (10). Furthermore, although ultrasonic irrigation instruments usually possess a noncutting design, they are made of a metal alloy that is harder than root dentin, and, therefore, their use risks changing root canal morphology. Consequently, Boutsioukis et al (10) suggested that PUI be replaced by ultrasonically activated irrigation.

In order to avoid the detrimental effects caused by ultrasonic activation, activating the irrigation solution with sonically driven noncutting plastic tips was suggested. Among the devices using this technique, the EndoActivator (Dentsply, Tulsa, OK) device appears to be the best-documented system (11). The principle is to use a polyamide tip to activate the solution and thus to prevent active cutting of the root canal walls or opening of the apical constriction. Passive sonic activation at low frequency was shown to be inferior to PUI with respect to bringing irrigation solution to the apex in variously tapered and curved canals (12). Furthermore, using simulated lateral canal models, no difference in cleaning efficacy was detected between PUI and passive sonic activation at low frequency (13). The maximum frequency of the aforementioned sonic irrigation system was measured to be 190 Hz (14).

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A novel sonic activation device has been developed that can be coupled to an air scaler that operates at 6000 Hz. The aim of the present study was to measure the bacteria-reducing effect of sonic activation at 6000 Hz in straight and curved root canals compared with PUI and manual irrigation.

Methods

We performed 2 experiments.

1. We tested the irrigation effect alone using 0.9% (w/v) sodium chloride as an irrigant in a short-term infection model.
2. We tested the additional effect of activation using 1.5% (w/v) NaOCl as an irrigant in a long-term infection model.

Experiment 1

Selection and Preparation of Teeth. For the experiment, we chose roots from maxillary premolars, palatal roots from maxillary molars, and maxillary front teeth. The teeth were obtained from a pool of extracted teeth stored in 1% chloramine solution. Informed consent was obtained from the donors. According to the local ethics committee, an approval for the irreversibly anonymized use of these bypass products is not needed.

The teeth were stored in 3% (w/v) NaCl in order to remove the periodontal tissue. After separating the roots, the curvature was radiographically measured in 2 planes according to the method of Schneider (15). The roots were grouped into curvatures <15° and >25° for further experiments. The working length was established using a size 10.02 file and was defined to be 0.5 mm shorter than the length after first apical visibility of the instrument. The roots were then negotiated with a reciprocating instrument size 25.08 (RECIPROC R25; VDW, Munich, Germany). The rinsing solutions were 3% NaCl and 17% EDTA in alternating mode. After closing the apex with composite (Telio CS Inlay; Ivoclar Vivadent AG, Liechtenstein), the roots were stored in deionized water until further use.

Microbial Contamination. Microbes were precultured for 18 hours and then suspended.

Before the experiment, the roots were placed in water and autoclaved (121°C, 20 minutes [Laboklav ECO; SHB Steriltechnik, Detzel Schloss, Germany]). After sterilization, the roots were inoculated for 3 days at 37°C under the conditions described later with 8 different microbes or combinations of microbes:

1. *S. gordonii* American Type Culture Collection (ATCC) 10558
2. *A. oris* ATCC 43146
3. *Fusobacterium nucleatum* ATCC 25586
4. *S. gordonii* ATCC 10558 and *A. oris* ATCC 43146
5. *S. gordonii* ATCC 10558 and *F. nucleatum* ATCC 25586
6. *E. faecalis* ATCC 29212
7. *Candida albicans* ATCC 76615
8. Clinical isolates obtained from a recurrent endodontic infection (6 species)

Groups 2, 3, 4, 5, and 8 were cultured under anaerobic conditions, group 1 with 5% CO_2, and groups 6 and 7 under aerobic conditions. The microorganisms were cultured for 18 hours on tryptic soy agar (TSA) plates with 5% sheep blood. The suspensions of *S. gordonii* ATCC 10558 were prepared in 0.9% sodium chloride (McFarland 4) and afterward diluted 1:9 with brain-heart infusion broth (Oxoid, Basingstoke, UK) supplemented with 5 mg/L β-nicotinamide adenine dinucleotide (Sigma-Aldrich, St Louis, MO). The suspensions of *F. nucleatum* ATCC 25586 and *A. viscosus* ATCC 43146 were prepared directly in Wilkins-Chalgren broth (Oxoid) with β-nicotinamide adenine dinucleotide. Anaerobic bacteria and streptococci were mixed at a ratio of 9:1.

Sterility was monitored at the beginning of the experiment with microbial sampling and subsequent culturing on TSA under respective conditions. The nutrient broth was renewed every day.

Irrigation Protocols. The irrigation solution contained 0.9% (w/v) sodium chloride. For each bacterial group, 10 roots were used in each of the 4 following irrigation protocols, which consisted of 5 straight roots and 5 curved roots:

1. Test group (n = 10): passive sonic activation (EDDY polyamide tip, VDW), 6000 Hz, coupled to an air scaler (SonicFlex, intensity mode III; KaVo, Biberach, Germany), 3 × 20 seconds
2. Positive control (n = 10): passive ultrasonic activation (IrriSafe + VDW Ultra, VDW), 3 × 20 seconds, power set at 20% according to the manufacturer's instructions
3. Negative control 1 (n = 10): manual irrigation (30-G endo-irrigation needle KerrHawe SA, Bioggio, Switzerland), 6 mL
4. Negative control 2 (n = 10): no treatment

In irrigation protocols 1 and 2, the roots were irrigated with 1 mL NaCl solution between the activation periods, and 1 mL NaCl solution was used as a final rinse. For irrigation protocols 1 and 3, the instrument/irrigation needle was placed at the working length; for irrigation protocol 2, the instrument was placed 1 mm short of the working length.

Counting of Microorganisms. Sterile paper points (R25, VDW) were placed for 30 seconds in the roots. These paper points were then transferred into sterile tubes filled with 1 mL NaCl solution. After intensive vortexing for 20 seconds and exposing to ultrasonication, 0.1 mL of the solution was dispersed onto an agar plate. After incubation at the respective conditions for 2 days (aerobes) or 7 days (anaerobes), colony-forming units (CFUs) were counted.

Experiment 2

In contrast to the setup described previously, we did not differentiate between curved and straight root canals in the second experiment. We tested groups 4 to 7 in a long-term infection model. The duration of incubation was 21 days under the same conditions as described previously. The irrigation protocols were the same as those described earlier, with n = 6 for each irrigation protocol. However, the irrigation solution in this part of the experiment was 1.5% (w/v) NaOCl followed by 1 mL NaCl solution.

In order to control sterility throughout the entire duration of the experiment, microbial samples were collected twice a week, cultured for 48 hours at 37°C on TSA, and checked for contamination.

Immediately after irrigation, a microbiological sample was taken as described previously. For 7 days, the root canals were filled again with nutrient broth, and they were incubated under the respective conditions at 37°C. After 3, 5, and 7 days, microbiological samples were taken from the root canals. We performed a qualitative dichotomic analysis confirming the presence or absence of bacteria after 0, 3, 5, and 7 days of reincubation.

Statistics. All statistical results were calculated using R 3.1.0 (R-project; University of Vienna, Vienna, Austria) or SPSS 23 (IBM, Armonk, New York).

For the first experiment, normality was checked by computing QQ plots as well as P values according to the Shapiro-Wilk test (P = .0004, indicating non-normality). Therefore, nonparametric methods were applied.

The goal was to compare the $\log_{10}$-scaled number of microorganisms after different treatment procedures. Secondly, the influence of the